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Pharmacogenomics: The Implementation Phase

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ABSTRACT Pharmacogenomics makes use of genetic and genomic principles to facilitate drug discovery and development, and to improve drug therapy. Its goal is to attain optimal therapy for the individual patient. This article analyzes current trends in pharmacogenomics and asks how this new science affects drug development in the pharmaceutical industry and the clinical use of drugs.

INTRODUCTION

Pharmacogenetics is a well–established discipline, which studies the genetic basis of interindividual variability in the response to drug therapy.[1-4] With the advent of genomic sciences, a paradigm shift has occurred from the study of individual genes and their corresponding proteins to an analysis of the whole: the genome, the transcriptome, the proteome, the metabolome, and further derivatives of –omics (cellome, phenome; create your own –omics specialty!). In contrast to the pervasive analytical–reductionist approach, genomics represents an integrative approach—an attempt to put all the pieces back together. Medical genetics and pharmacogenomics take center stage in this (re)emerging science philosophy.

The current transition from pharmacogenetics to pharmacogenomics is compelling. Over the previous five decades, numerous studies have revealed the important contribution of variant genes to interindividual variability in drug response. Yet, it has become apparent that each drug interacts with numerous proteins in the body (transporters, enzymes, binding proteins, and receptors), and moreover, affects hundreds of proteins in metabolic and signaling pathways downstream of the primary interaction. Pharmacogenomics provides an approach to resolve such complex systems. Here, I use the term pharmacogenomics to include all –omics disciplines as applied to drug therapy. This avoids the use of derivative terms such as pharmacoproteomics, or 'functional' genomics—the latter introduced primarily by the biotech industry once funding for 'pure' genomics ran dry.

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LONG-RANGE GOALS OF PHARMACOGENOMICS

Medical genetics aims at understanding health and disease from a molecular genetic perspective, and one of its main goals is to identify disease susceptibility genes. In contrast to single–gene Mendelian disorders (eg, cystic fibrosis), most major diseases are multigenic, and hence, many gene variants affect disease processes, with varying penetrance. Over 250 genes have been identified for cardiovascular diseases (http://www.tcgu.med.utoronto.ca/tcgu/overview.html).[5,6] It is understood but has yet to be proven that the spectrum of variant genes contributing to cardiovascular diseases has a significant impact on an individual's risk and disease progression. On the other hand variant genes can also determine the outcome of drug therapy, a subject area of pharmacogenomics. Some understand pharmacogenomics to provide the means for drug selection in the following sense:

- The right drug for the right disease.

In contrast, pharmacogenetics is seen to optimize an individual's therapy by focusing on proteins (and the respective gene variants) directly interacting with the drug (e.g., CYP450). In other words:

- The right drug for the right patient.

This distinction is specious as genes could fall into both categories—for example those encoding receptors. The overriding goal is to optimize therapy of the individual patient with the use of genetic–genomic information. Whether a gene affects the drug response directly or lies downstream of drug effects does not represent a fundamental distinction.

In the early excitement about unraveling the human genome, expectations for pharmacogenomics soared. Here are some of the lofty visions of how medicine and therapy will look like in the (not too distant) future.

- Most susceptibility genes will be known for all major diseases. Individuals will carry a gene chip with information on all relevant gene variants that serve to guide proper life styles and therapy.

- The gene chip will also contain an individual's genetic background relevant to drug therapy (e.g., genes involved in transport and metabo-

lism) which further aids in the selection and dosage of drugs.

- Finding numerous disease susceptibility genes will lead to the discovery of hundreds, if not thousands of new drug targets, and hence, a wave of drug discovery.

- Genetic profiling may permit early intervention or even prevention of disease—arguably the most desirable goal.

WHAT CAN WE IMPLEMENT AT PRESENT. A REALITY CHECK

The underlying assumption is that genetic factors play a key role in disease and therapy. One might argue that environmental, cultural, and other factors such as age and nutrition, may outweigh genetic factors in many cases—in the still ongoing debate about nature versus nurture. However, we cannot separate environmental and genetic factors, and rather must ask how they interact with each other to determine disease and therapeutic outcome. Therefore, genetic factors represent only one aspect of disease management (or maybe rather health management) to be added to all medical and pharmaceutical information currently used in therapeutic decisions. Even if we understand in quantitative terms what impact a given gene variant has on the response to a given drug, genotyping all patients prospectively may not provide the optimal answer, for the following reasons.

- The genotype may not predict the phenotype in each case. A hallmark of complex systems is its unpredictability. The less certain the prediction, the less benefit can be derived from a therapeutic decision based on genotype.

- Even if genotyping becomes mandatory in conjunction with a specific drug therapy, legal, ethical, and economic factors impact the risk–benefit and cost–benefit ratios. Genotyping may not occur in each case, thereby exposing these individuals to greater risk of adverse effect. Therefore, multiple factors play into the decision whether genotyping should become standard praxis. These include lack of response where it has severe consequences (AIDS therapy), and a high frequency and severity of adverse effect, if there are no alternative drugs available.[7]

However, therapeutic benefits can emerge from genetic information alone, without the need to genotype the patient to be treated. For example, when prescribing codeine, a physician or pharmacist should know the frequency of CYP2D6 poor metabolizers (2D6 activates codeine to morphine) in the patient population, ranging from 2–10% dependent upon ethnicity. The patient should be advised that some individuals do not respond to codeine, and to report back immediately if pain is not alleviated. Therapy should then switch to a narcotic analgesic that does not require metabolic activation by CYP2D6 (the more potent oxycodone certainly does and should not be substituted for codeine). Yet, even this seemingly straight forward example brings out further complexities of the therapeutic process. Pain therapy includes placebo effects as an integral part of the response. Could the placebo effect vanish or even work in the opposite direction in some patients who are advised that the drug may not work for them?

Professional education and careful monitoring can go a long way in optimally utilizing genetic information for therapy. Currently, there is much interest from prescribers to learn more about pharmacogenomics, but little opportunity to make the available information useful to health care professionals and patients alike. We need to address and overcome this deficit.

The expectation that we soon will have new drug therapies against all kinds of illnesses, even those that are currently untreatable, may have to be tempered. The search for blockbuster drugs—effective against major diseases in large patient populations—has proven difficult to say the least. Drug development costs now approach US $1 billion! Possibly, we have misunderstood the lessons gained from genomic sciences—living organisms are extremely complex systems that rarely respond in a predictable fashion if one of its countless components is disturbed (i.e., the drug target). The broader the patient population to be treated, the greater the chances that some patients will experience adverse effects or will not respond.

On the other hand, pharmacogenomics can serve to subdivide a patient population into several groups based on genotype. Within each subgroup, drug effects may become more predictable so that the risk/benefit ratio is reduced. Moreover, assessment of disease susceptibility and progression of disease could also become more reliable. This would permit more aggressive therapy where indicated or initiation of therapy at the earliest symptoms of disease when drugs might well prove more effective—at lower doses and with fewer side effects. Therefore, more realistic objectives of pharmacogenomics should be to:

- Develop a structured knowledge base that is readily accessible to health care professionals to aid in optimizing drug therapy. Similarly, patients should have access to genetic information presented such that it is understandable for laypersons.

- Define patient populations by SNP patterns (haplotypes; see below). This approach could replace classification by ethnic groups which are

often highly heterogeneous, providing an objective foundation for population analysis on the basis of genomic variability and dynamics.

- Define conditions under which prospective genotyping of individual patients is advisable as integral part of therapy.[7]

- Develop guidelines for the application of pharmacogenomics during drug development and phase I–III clinical trials, and post–approval monitoring.

- Develop in vitro tests to link genotype with phenotype—for example by measuring drug effects in target tissues from multiple individuals.

- Identify conditions and diseases where early intervention and prevention can occur on the basis of genetic information.

The first objective is to disseminate knowledge in such a way that it can affect clinical praxis. We must reduce current knowledge to a level where it can have immediate impact on therapeutic decisions. Clearly, ability to predict disease susceptibility and therapeutic outcome with sufficient accuracy is critical. Whereas the relevance of genetic factors is already established in some instances, it is equally important to understand where current knowledge is limited. An easily accessible databank could reduce the level of anxiety and resistance among health care practitioners as to how and when to use genetic information.

Few drug therapies currently involve prospective genotyping, and in no case is it mandatory. Examples include intensive anti–AIDS therapy (HAART)[7] where close to one half of all patients are being tested for their HIV genotype to exclude resistant drugs from the regimen. In the case of thioguanine therapy of childhood leukemia, thiopurine methyltransferase (TPMT) is required to inactivate the drug. As a result, patients with two non-functional TMPT alleles will suffer severe toxicity from thioguanine unless the dose is drastically reduced.[8] However, the incidence of homozygous patients lacking functional TPMT is only 0.3%, requiring the screening of 300 patients before the thioguanine dose can be reduced prospectively in a single patient. While this could prevent serious side effects in such a special patient, it also adds considerable cost to thioguanine therapy. An alternative approach would be to monitor all patients carefully for early signs of thioguanine toxicity and stop treatment immediately. Thus, proper application of genetic knowledge alone could reduce the severity of adverse effect. What is needed now is to establish the cost-benefit ratio, using principles evolving from the field of pharmacoeconomics.[7] Strict criteria need to be developed to determine whether prospective genotyping is suitable as part of therapy. In the case of thioguanine, this question has yet to be fully resolved.

Drug development could substantially benefit from early inclusion of pharmacogenomics. This might alert one to possible adverse effects in target populations, or it could serve to identify responsive patient populations most likely to benefit from therapy. The latter is particularly attractive because it enables one to target a drug to a well defined disease phenotype that can later be expanded to include additional indications as warranted. This has the potential to reduce development costs substantially.

The use of in vitro assays employing target tissues from multiple individuals is a promising approach. Currently, a limiting factor in pharmacogenomics is the link between genotype and phenotype—such as therapeutic outcome, which is far removed from the immediate actions of a variant gene. Yet, target tissues are not readily available from the patient under treatment. To overcome this deficit, one could take advantage of currently emerging stem cell technology, using either embryonic or adult stem cells to capture the main genotypes in a patient population and study drug effects on the target tissue in the context of an individual's genetic background. For example, drug-induced long QT syndrome as a potential cause for drug failure (even after the drug has been approved) could be detected by studying drug effects on contractility of cardiac myocytes grown from stem cells of >100 individuals. This permits the association of drug effects with specific polymorphisms of ion channel genes already known to be involved in long QT syndrome, modified by the genetic background of a sufficient number of individual subjects. The information gained from such an approach could be valuable in detecting potential adverse effects very early in drug development.

Alternatively, one could exploit adult stem cells extracted from a patient to test drug effects in vitro to optimize this patient's therapy. Adult stem cells derived from parenchymal tissue, bone marrow, or skin, can be (trans)differentiated into a spectrum of target tissues, including neurons, myocytes, hepatocytes, and endothelial cells. While this technology is still emerging for the purpose of growing large quantities of target tissues from adult stem cells for tissue replacement therapy, the cell yield is sufficient for diagnostic purposes. I predict important applications for this approach.

As to prevention and early treatment, this must become an area of high priority. Yet, we are poorly prepared for it, with a majority of resources going towards the treatment of complex diseases in the elderly—the most likely target for 'blockbuster drugs'. Advances in the treatment of these chronic disorders are difficult to achieve, as evidenced by the difficulty in getting novel drugs approved. Whereas early treatment and prevention could be highly effective even with existing drugs, it calls for inexpensive therapies with minimal adverse effects—after all the pa-

tient would have to be treated over much longer time periods with little or no symptoms apparent. An example is the use of 'baby' aspirin in preventive treatment of coronary artery disease. Note that baby aspirin usually has a sherry flavor to make its use palatable to children—where is the convenient and inexpensive low-dose aspirin for adults? Currently, there is no incentive for the pharmaceutical industry to pursue early therapy and prevention, particularly if testing of off–patent drugs is involved. We must find a way to overcome this glaring gap in our approach to improve therapy and health maintenance and provide the requisite incentives to explore the value of early therapy and prevention.

CURRENT APPLICATIONS OF PHARMACOGENOMICS

Adverse drug effects. Adverse drug effects have been associated with genetic factors but quantitative assessment of the role of variant genes has been lacking. A study by Lazarou et al.[9] suggested that fatalities resulting from adverse drug reactions (ADRs) represent a leading cause of death in the USA. This type of ADR was considered 'unavoidable' as the drug was given in the proper dosage to the patients with appropriate indications.

To determine whether and to what extent genetic variability might account for ADRs, we have performed a critical review of the literature on ADRs and pharmacogenetics. Our results indicate that ~60% of the main drugs causing ADRs are metabolized by one or more cytochrome P450 enzymes with a high frequency of inactive alleles. 'Poor metabolizers' are patients who carry two nonfunctional alleles of the responsible CYP gene.[10] In contrast, randomly selected drugs failed to show a strong association with polymorphic CYP genes. The highly polymorphic CYP2D6 was implicated in a sizeable portion of the adverse reaction drugs (~30%), as expected. Indeed, metabolism by CYP2D6 is of concern if discovered during drug development since up to 10% of patients are poor metabolizers. In this group, one would expect a higher incidence of adverse drug effects. Therefore, pharmaceutical companies tend to eliminate CYP2D6 substrates as drug candidates. However, we also found that CYP2A1—metabolizing only 5% of randomly selected drugs—is involved in the metabolism of a majority of drug in the adverse reactions panel. Since CYP2A1 poor metabolizers can reach 15% in a patient population, this also needs to be considered in drug development and therapy. Hence one might consider removing CYP2A1–interacting drug candidates from further development as well.

On the other hand, very few functional polymorphisms are known for the main metabolizing enzyme CYP3A4, responsible for the metabolism of over 50% of all drugs. Yet, CYP3A4 activity in the liver is highly variable among patients, even in the absence of known mutations—possibly because mutations reside in yet unrecognized promoter regions or in genes encoding transcription factors regulating CYP3A4 expression in trans. As a result, we should be cautious in rejecting drug candidates just because they happen to metabolized by a polymorphic CYP enzyme. What really counts is the variability of metabolizing activity in the patient population, regardless of the cause, and the potential for adverse effects. Nevertheless, our study linking adverse drug effects with genetic polymorphisms suggests that current genetic information if properly applied in therapy might be able to reduce the incidence of ADRs. Once our knowledge base expands, more compelling applications are likely to arise.

Drug efficacy. Genetic variations that affect pharmacodynamics involve genes that either interact directly with the drug or contribute to the disease process per se.[2-4] For example, lipid lowering drugs are important in the therapy of coronary artery disease (CAD). Specifically, HMG-CoA reductase inhibitors—also referred to as statins—are widely used but appear to be ineffective in one out of five patients to prevent disease progression. Recently, numerous genetic variations predisposing to or associated with atherosclerosis and CAD have been proposed.[5,6] Common genetic variations occur in genes encoding apolipoproteins, cholesteryl ester transfer protein (CETP), lipases, coagulation factors, endothelial nitric oxide synthase, and others. CETP activity is inversely related to HDL levels, and CETP polymorphisms thus may affect CAD. Kuivenhoven et al.[11] have studied the effect of CETP genotype on CAD progression and response to pravastatin. Their findings suggest that pravastatin efficacy varies with CETP genotype in Caucasian males. Another study with pravastatin suggested an association between ApoE alleles and therapeutic response. Moreover, a variant of lipoprotein lipase (LPL) was shown to modulate adverse metabolic effects of treatment with beta–blockers. Genetic variations of CETP, apolipoproteins, and LPL may also determine part of the response to dietary intervention. Thus, multiple genetic markers have been implicated as possible predictors of CAD treatment. Until now, it has not been possible to define statin non–responders in genetic terms with sufficient accuracy to affect actual therapy.

HAPLOTYPE ANALYSIS AND PATIENT POPULATION ANALYSIS

The study on cardiovascular disease and statin therapy by Kuivenhoven[11] has relied on a single SNP marker in an intronic region of CETP. This raises the question which functional variant is in linkage disequilibrium with this marker–requiring the determination of haplotype distribution in the population (multiple phased SNPs on the same chromosome).[6] Drysdale et al.[12] have demonstrated that the haplotype, rather than single SNPs, of

the b2 adrenoceptor gene determines the response to albuterol in asthmatic children, clearly establishing the need for haplotype analysis. On the other hand, Arranz et al. [13] introduced the use of multiple candidate genes—but only single polymorphisms rather than haplotypes—to predict the response to clozapine in the antipsychotic therapy.

A compelling but complex step forward will be the use of multiple candidate genes combined with haplotype analysis for each gene. Recent advances in understanding haplotype blocks in the human genome [14,15] have shown that a surprisingly small number of haplotypes account for a majority of patients in diverse populations. Just three haplotypes often describe variations among 80% of the human population. Moreover, haplotype blocks are larger than expected, extending over 50–100 kb in Caucasians – considered a bottleneck population because it has arisen from a rather small pool migrating from Africa to Europe. On the other hand, an African population displayed much larger diversity with smaller haplotype blocks (~5 kb), indicative of an older population with more diverse subgroups.[14] One can readily appreciate that the dynamic evolution of the human genome has major impact on the study design, as a function of the patient population.

We have begun a study on statin therapy and coronary artery disease progression, based on haplotype analysis involving multiple candidate genes. It remains to be seen whether the results will permit us to identify responders and non–responders with sufficient accuracy for therapeutic applications. Having reliable criteria to identify non–responders might at first appear to be a pyrrhic victory as there are no clear alternatives for therapy to the rather effective statins. Yet, such a well defined patient population would provide the needed incentive for new drug discovery, providing well defined endpoints and patient populations. It is also possible, however, that our study will reveal an inability to predict therapeutic outcome with this type of approach, because the complexity of the systems swamps out the impact of genetic predictors. Such a negative outcome is equally important as alternative therapeutic strategies need to be developed to optimize individual therapy. Each disease, each drug, and each patient population may present unique problems to the therapist.

HOW DOES–OR HOW SHOULD– THE PHARMACEUTICAL INDUSTRY REACT TO PHARMACOGENOMICS?

Decision makers in the drug industry may ask what financial advantage pharmacogenomics holds for their product, whether already in clinical use or under development. Since the answer is mostly uncertain, any incentive to pursue pharmacogenomics dissipates easily. A pharmacogenomic study could result in a smaller target population, economically an entirely undesirable effect. Understandably, one is reluctant to invest in the science, particularly concerning established drugs already in use. On the other hand, pharmacogenomic information could actually enhance market share, if an effective drug is only second or third choice because of adverse effects, and the genotyping could avert the adverse effects. In the long run, however, it is compelling to identify the patient population least likely to experience adverse effects and most likely to respond favorably. As demonstrated with the statin example, only 20% of the patients realize little therapeutic benefit from statins, leaving 80% of patients that can be treated effectively.

Pharmacogenomics could lead to numerous new therapies applicable to relatively small and well defined patient populations. With current costs for new drug development skyrocketing, however, incentives are lacking to bring such drugs to market. Yet, we must find a way to reverse the trend towards mega–blockbuster drugs with ever increasing up–front costs because it is unsustainable in the long run. Regulations providing orphan drug status already provide incentives for developing drug therapies aimed at small patient populations. Early application of pharmacogenomics to identify the target population most likely to benefit could further reduce the cost by reducing the number of required subjects in clinical trials—even though actual examples to support this are lacking. Reversing the trend from blockbuster drug to more narrowly targeted drugs with well defined patient populations may well turn out to be a necessary change for the pharmaceutical industry. Whether genotyping of individual patients as part of therapy will gain acceptance in the near future remains doubtful. In many cases, effective use of genetic–genomic information together with all other clinical data might suffice to optimize drug therapy.

In conclusion, pharmacogenomics compels us to approach drug therapy in novel ways, with focus on optimal therapy of an individual patien or a well defined patient population. It is likely to usher in a new era promising profound changes in drug therapy.

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For a guided literature survey, go to the Virtual Journal in Pharmacogentics–Pharmacogenomics in AAPS PharmSci, click on enter, and perfom literature searches of your area of interest with carefully selected PubMed mesh words. You can also modify or expand the search terms to cover a more focused area.

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